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High-Performance Liquid Chromatographic/Mass Spectrometric Determination of Volatile Carboxylic Acids Using Ion-Pair Extraction and Thermally Induced Alkylation

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High-Performance Liquid Chromatographic/Mass Spectrometric Determination of Volatile Carboxylic Acids Using Ion-Pair Extraction and Thermally Induced Alkylation

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Low molecular weight volatile carboxylic acids have been analyzed by high-performance liquid chromatography/mass spectrometry (HPLC/MS) using post-column derivatization and a moving belt interface. In order to prevent loss of the volatile solutes during transport to the mass spectrometer, the acids were ion paired with tetrabutylammonium counterions. Flash vaporization of the ion pairs into the mass spectrometer yielded butyl esters which were analyzed by chemical ionization MS. Aqueous samples containing the free carboxylic acids were analyzed by direct injection into the liquid chromatograph. Detection limits in the parts-per-billion range were achieved.

Short-chain volatile carboxylic acids are important indicators of a wide variety of bioorganic processes. For example, in a mammalian system the organic acid profile may be helpful in the identification of healthy states or inborn errors of metabolism (*1*). Their identification and quantitation are also important in a variety of microbiological and dental applications (*2–4*). From an environmental point of view, the analysis of low molecular weight carboxylic acids is necessary in the study of bacterial degradation in sewage treatment systems (*5*). These acids are produced during the anaerobic digestion of proteins and carbohydrates; when allowed to accumulate too rapidly, they can kill methane-producing bacteria and eventually stop the digestion process.

The trace-level analysis of low molecular weight organic acids in biological fluids is normally conducted by gas chromatography (GC) or combined gas chromatography/mass spectrometry (GC/MS) (*6*). A major limitation of these approaches, however, is the isolation of the acids from the substance prior to analysis by the indicated method(s). Typically, the recovery process may involve batch extraction, steam distillation, anion exchange chromatography, and other related techniques (*6*). Vacuum distillation for recovery of the acids from biological materials has also been utilized (*7*). All of the standard methods for volatile acid analysis in sludge provide only a total acid value rather than specific results for individual acids (*8*). In addition, the silicic acid column chromatographic method (504A) has interferences from dibasic acids; the steam distillation method (504B) is time-consuming; and the direct distillation method (505C) is of limited accuracy. Finally, the development of the master analytical scheme, a protocol that was reliable for GC/MS analysis of most carboxylic acids in industrial effluents, gave poor reproducibility of recoveries of the most volatile acids (*9*).

It is likely that many of the problems of poor reproducibility are derived from the elaborate sample recovery procedures required to prepare a sample for vapor-phase analysis by GC or GC/MS. On the other hand, much of the inconvenience associated with sample preparation can be overcome by using HPLC/MS, in which case the HPLC provides both the necessary separation power and the sample cleanup. In view of this inherent advantage offered by HPLC and the general potential of HPLC/MS for trace-level analysis due to the high sensitivity and selectivity of the mass spectrometric detector, we investigated the utility of HPLC/MS for the analysis of these volatile compounds.

It is interesting in this context to note that we are accustomed to thinking of HPLC/MS as a technique for the analysis of nonvolatile and/or thermally labile compounds. The example described in this paper, however, illustrates the flexibility of HPLC/MS and the diversity of problems that can be addressed by judicious selection and adaptation of chemical procedures to accommodate the given interface and sample on hand. Our approach relies on the use of the moving belt interface using tetrabutylammonium counterions for a post-column ion-pair extraction followed by alkylation on the belt.

This study focused on the analysis of the four lowest molecular weight *n*-alkyl carboxylic acids: formic, acetic, propionic, and butyric. In the following we present the basic strategy and approach to this problem using standards, and we conclude with an example showing the effectiveness of this approach for the analysis of three samples of tap water spiked with a mixture of the first three acids.

EXPERIMENTAL SECTION

Reagents. All solvents were HPLC grade purchased from J. T. Baker Co., Phillipsburg, NJ. Tetra-*n*-butylammonium hydroxide was purchased as a 40 wt % solution in water from Aldrich Chemical Co., Milwaukee, WI. Solutes and buffers were of analytical quality, purchased from Fisher Scientific, Inc. (Fairlawn, NJ), and were used without further purification.

Apparatus. *HPLC.* Chromatographic separations were conducted isocratically on a 150 × 4.6 mm i.d. C_8 column packed with 5-μm Hypersil silica gel (Whatman, Clifton, NJ). The mobile phase was 95:5 H_2O/acetonitrile and the flow rate was 1 mL/min. All pumps (P_1, P_2, P_3) of Figure 1 were Waters Associates Model 6000A. The injection valve was a Valco air-actuated, V6-UHPa-N60 valve (Valco Instruments, Houston TX). HPLC/UV analysis was conducted with an LDC Model 1205 UV detection at 215 nm.

Post-Column Reaction System. The post-column reaction system, shown in Figure 1, consisted of two mixing tee's, an extraction/reaction coil, and a phase separator. The mixing tee's were commercial low dead volume union tee's (Swagelok, part no. SS-1FO-3GC). The extraction coil was of 1 m length and 0.8 mm i.d. The phase separator was constructed from a glass tee (Technicon, Tarrytown NY) part no. T 1160522-01A. A tube made of Teflon ($^1/_{16}$ in. o.d., 0.8 mm i.d.) was placed within the glass tee to provide a wetting surface for the methylene chloride extracting solvent.

Mass Spectrometry. A Finnigan 4021B mass spectrometer interfaced to an INCOS data system was used. A moving belt interface was used for HPLC/MS coupling. The mass spectrometer was operated in the chemical ionization mode with isobutane as reagent gas at an ion source temperature of 200 °C and indicated pressure of 0.2 torr.

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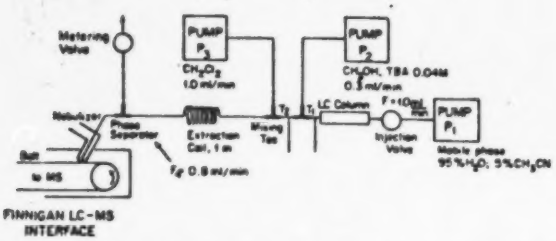


Figure 1. Schematic diagram of HPLC/MS system showing post-column column reaction/extraction interface. Details are given in the Experimental Section.

Scheme I

$$RCOO^-N(CH_3)Ph \rightarrow RCOOCH_3 + (CH_3)_2NPh$$

RESULTS AND DISCUSSION

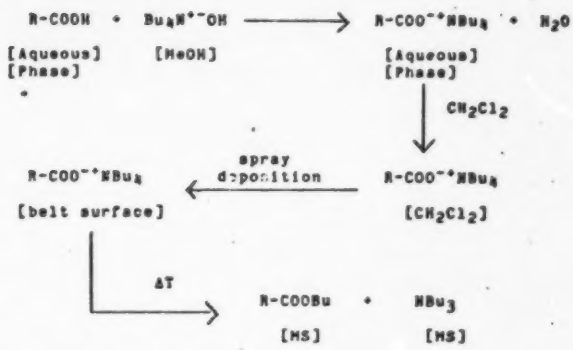
Strategy of Analysis. The analysis of low molecular weight (~100) volatile compounds by HPLC/MS represents a major challenge because that range of the mass spectrum is rich in ion current arising from the mobile phase solvent and its additives. In direct introduction methods, such as the direct liquid introduction (DLI) or the thermospray, this problem may be further amplified by the occurrence of solvent ion clusters that may extend into the mol wt 100 range (*10*). Even with the moving belt interface, the solvent is not totally removed as the belt passes under the IR heater and through the vacuum locks prior to entry into the mass spectrometer. For the direct analysis of volatile compounds such as formic or acetic acid, the moving belt interface suffers from the additional disadvantage that the analytes themselves are more than likely to be removed during the solvent vaporization steps. On the other hand, as we have shown previously (*11–13*), the moving belt interface can be readily coupled to a post-column reaction system in order to render the short-chain carboxylic acids nonvolatile, thus permitting their safe transport through the vacuum locks. Concurrently, chemical derivatization can increase the molecular weight of the compounds, thus yielding spectra in a mass range relatively free of background interferences. In this paper, the approach employing this strategy relied on the principle of ion-pair formation and pyrolytic alkylation.

We have previously reported that long-chain fatty acids such as stearic acid or oleic acid can be converted to the corresponding methyl esters upon pyrolysis of trimethylanilinium ion pairs on the flash vaporizer of the moving belt interface (*13*) (Scheme I).

This pyrolytic alkylation is analogous to alkylation reactions carried out in the injection port of a gas chromatograph during the GC or GC/MS analysis of carboxylic acids (*14*). Since the mass increment introduced by methyl ester formation is not sufficient to raise the molecular weight of the analytes beyond the range of spectral interferences, we explored the incorporation of progressively larger alkyl groups. The thermal degradation of the carboxylate/tetrabutylammonium ion pair provided an acceptable compromise in terms of the facility of alkyl group transfer, extractability into an organic solvent, and the formation of esters with a molecular mass relatively free of background interferences.

As shown in Figure 1, methanol containing 0.04 M tetrabutylammonium hydroxide was mixed with the aqueous mobile phase after the column. The carboxylic acids were extracted into a CH_2Cl_2 organic layer added at T_2 (Figure 1) as the tetrabutylammonium ion pairs and then transferred onto the belt surface. Deposition of the eluent onto the belt occurred by nebulization in order to minimize band broadening (*15*). By this approach, the carboxylic acids were rendered nonvolatile and were thus immobilized on the belt surface during the removal of the methylene chloride. The *n*-butyl esters of the acids were then formed by pyrolytic dehydration of the ion pairs at the flash vaporizer. The sequence of reactions is outlined in Scheme II.

Scheme II. Reaction Sequence for Derivatization of Carboxylic Acids



HPLC/MS Analysis. The chemical ionization (isobutane) mass spectra of the *n*-butyl esters of the four lower carboxylic acids exhibit abundant protonated molecular ions at m/z 103 (butyl formate), m/z 117 (butyl acetate), m/z 131 (butyl propionate), and m/z 145 (butyl butyrate). These ions were by far the most dominant features of the CI (isobutane) mass spectra of the compounds. Surprisingly, under the conditions of the analysis, the mass spectrum of the acetic acid derivative was found to exhibit a significant peak at m/z 103 and a weaker one at m/z 131. To a lesser extent, the spectra of the other acids also exhibited peaks at m/z values coincident with those of the $(M + H)^+$ ions of the homologues in the series. It is not totally clear at present whether these spectral overlaps are due to impurities in the CI gas or other reagents. However, as shown in the HPLC/MS chromatograms of the acids, these peaks are most likely associated with the butyl ester formation upon flash vaporization of the ion pairs.

The protonated molecular ions of the *n*-butyl esters were used with selective ion monitoring to construct calibration plots and establish the detection limits for the four analytes. Fortuitously, at those m/z values, contributions from the CI spectrum of the ion-pairing reagent and the mobile phase were relatively minimal, as indicated by the background spectrum in the range from m/z 100 to m/z 250 in Figure 2. The base peak at m/z 186 corresponds to a protonated tributylamine ion.

A standard calibration curve (peak area vs. quantity injection into LC) for the four carboxylic acids, obtained at a flash vaporizer temperature of 250 °C, is shown in Figure 3. A linear dynamic range of nearly 3 orders of magnitude is indicated. For the most part, the detection limits were on the order of 10 ng, although in the case of propionic acid, whose *n*-butyl ester showed the highest response, a detection limit of 1 ng was achieved. As an example of low-level results, typical ion current profiles obtained at or near the detection limits for the four acids are shown in Figure 4. Ion current profiles for the protonated molecular ion of *n*-butyl acetate (m/z 117) obtained from varying amounts of acetic acid injected into the HP liquid chromatograph are shown in Figure 5. These profiles and the calibration plots were generated by injecting varying amounts of the acid solutes into an open tube instead of a packed column. Otherwise, the sequence of steps involving ion-pair formation, extraction, and esteri-

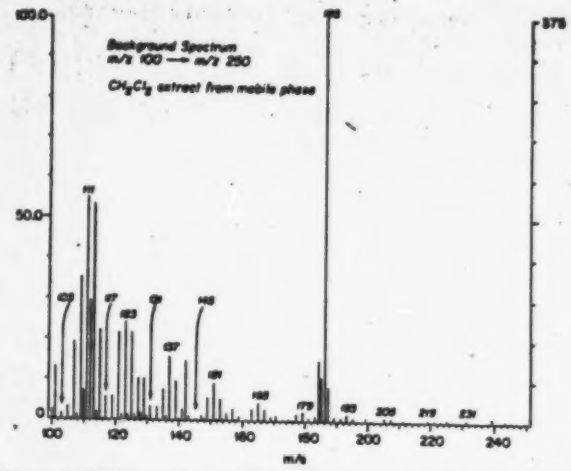


Figure 2. Background spectrum (CI isobutane) of methylene chloride extract from aqueous mobile phase.

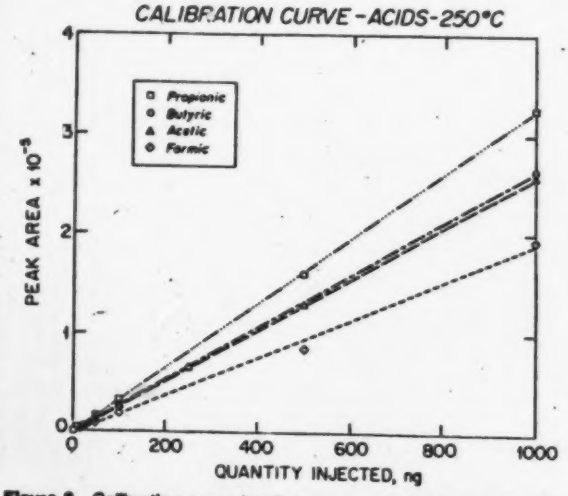


Figure 3. Calibration curve (peak area vs. quantity injected) of carboxylic acid butyl esters.

fication via flash vaporization was carried out as described above in Scheme II and Figure 1. The mass spectrometer was operated in the selective ion monitoring mode. Good run-to-run signal reproducibility with a standard deviation of less than 5% at the 100-ng injection level was observed in these determinations.

The three lowest molecular weight carboxylic acids—formic, acetic, and propionic—were then analyzed by reversed-phase HPLC/MS using a mobile phase of 95:5 H_2O/CH_3CN and a C_8 column. Figure 6 shows the HPLC/MS chromatogram of the compounds obtained from 1 μg of each test solute injected into the HP liquid chromatograph. In this case, the mass spectrometer was operated in the scanning mode in the range from m/z 100 to m/z 300. The lowest trace represents the total ion current, while the selective ion chromatograms of the respective molecular ions are presented in the three upper traces. The HPLC/UV profile is shown in the inset. It is apparent that despite the complexity of post-column ion-pair formation, extraction, and alkylation by flash vaporization, well-defined chromatograms can be obtained. In fact, band broadening measurements conducted in a separate series of experiments reveal that the contribution to peak variance from this series of steps using the apparatus of Figure 1 is minimal (*16*). In addition, it is important to point out the spectral overlap among the three analytes as reflected by the peaks

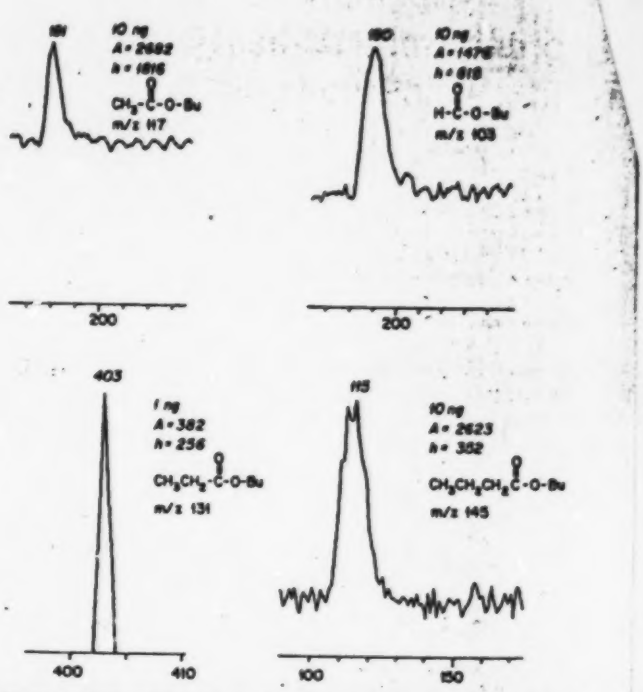


Figure 4. Protonated molecular ion chromatographic profiles of carboxylic acid butyl esters, showing signal-to-noise ratio at or near the detection limits. Areas and peak heights are given in A/D units.

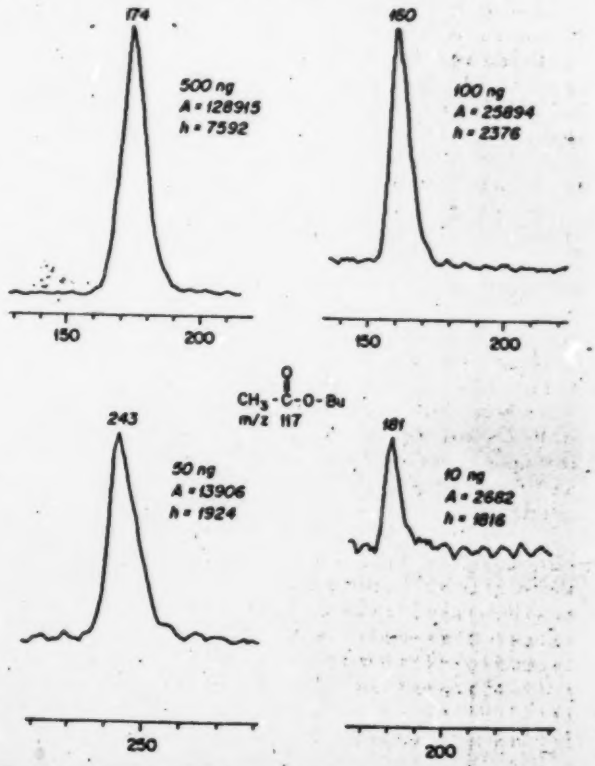


Figure 5. Protonate molecular ion chromatographic profiles of the butyl ester of acetic acid in the range from 500 to 10 ng of acetic injected into the HP liquid chromatograph. The mass spectrometer was operated in the simple ion monitoring mode. MS response is indicated in terms of peak area and peak height.

at m/z 131 in the spectra of butyl formate and butyl acetate, m/z 117 in the spectra of butyl formate and butyl propionate, and m/z 103 in the spectra of butyl acetate and butyl pro-

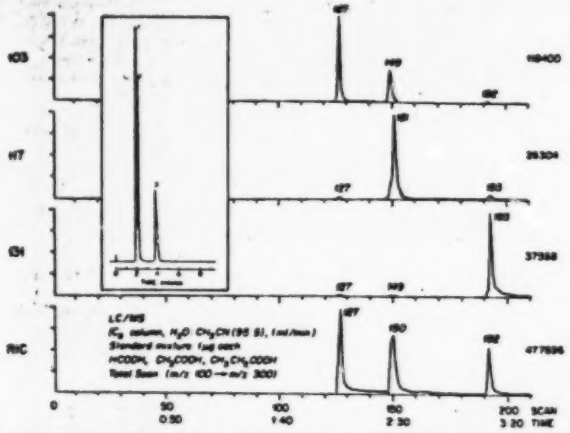


Figure 6. HPLC/MS chromatogram of standard mixture of 1 μg each of formic, acetic, and propionic acids. The mass spectrometer scanned in the range from 100 to 300 amu at a rate of 0.5 s/scan. Total ion chromatogram is shown in the bottom trace and mass chromatograms in the upper traces. Inset shows HPLC/UV chromatogram of the mixture. HPLC conditions are given in the Experimental Section.

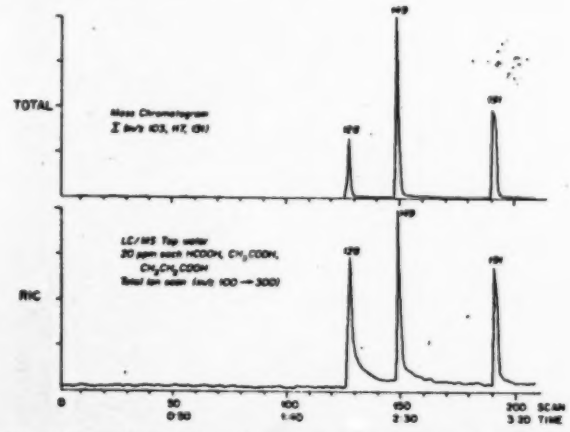


Figure 7. HPLC/MS chromatogram of tap water containing 20 ppm each of formic, acetic, and propionic acids. The mass spectrometer scanned in the range from 100 to 300 U at a rate of 0.5 s/scan. HPLC conditions are described in the text.

pionate. Significantly, the HPLC separation identifies the extent to which these peaks are associated with each compound. Similar chromatograms were obtained with standard mixtures containing as little as 10 ng of each acid.

Following the experimentation with standard acid mixtures, we examined a series of tap water samples spiked with levels of each acid corresponding to 20, 2, and 0.2 ppm. The aqueous samples were injected directly into the HP liquid chromatograph without any prior cleanup. In each case, a sample volume of 50 μL was injected, corresponding to 1000, 100, and 10 ng of each acid, respectively. Figure 7 shows the total ion HPLC/MS chromatogram from the 20 ppm sample. The top trace represents the summation of the ion currents of the protonated molecular ions of the three esters. Figure 8 shows the results from the analysis of the 2.0 ppm sample while the HPLC/MS chromatogram from the 0.2 ppm sample is given in Figure 9. In the latter case, the mass spectrometer was operated in the multiple ion detection (MID) mode where only the three protonated molecular ions were monitored. The 50-μL injection represented the introduction of 10 ng of each acid into the system shown in Figure 1. The results of Figures 7 and 8 clearly indicate the feasibility of the proposed approach of post-column ion-pair formation, on-line extraction,

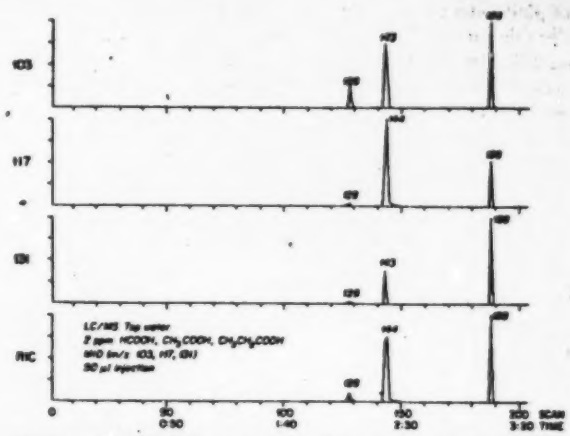


Figure 8. HPLC/MS chromatogram of tap water sample containing 2.0 ppm each of formic, acetic, and propionic acids. Conditions are as described in Figure 7.

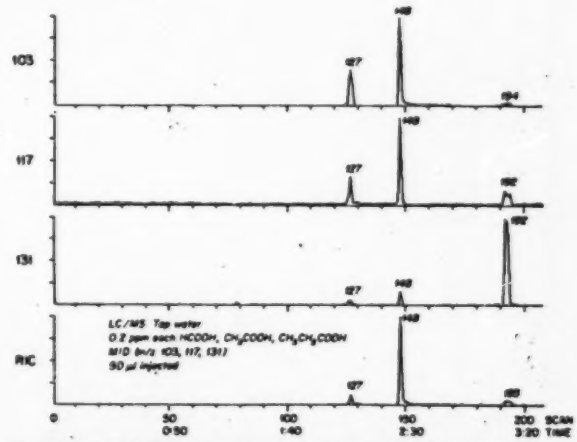


Figure 9. HPLC/MS chromatogram of tap water sample containing 0.2 ppm each of formic, acetic, and propionic acids. The mass spectrometer was operated in the multiple ion detection mode (m/z 103, 117, and 131).

and alkylation by flash vaporization for the analysis of low molecular weight carboxylic acids.

CONCLUSIONS

The use of HPLC/MS for the analysis of low molecular weight, volatile compounds has been demonstrated. Post-column chemistry adds flexibility and versatility to on-line HPLC/MS and improves their compatibility (*17*). The HPLC/MS analysis of low molecular weight carboxylic acids via ion-pair extraction and thermal alkylation offers the following advantages: (1) Ion pairing prevents premature vaporization off the belt with the solvent. (2) Butyl ester formation provides a mass increment raising the molecular weight of the acids beyond the range of major spectral interference. (3) Post-column extraction into CH_2Cl_2 reduces background contribution from the aqueous mobile phase. (4) Analysis of aqueous samples is possible by direct injection into the HP liquid chromatograph without prior cleanup. As is apparent from the results detailed above, analysis in the parts-per-billion range is well within the capabilities of the procedure. Currently, a limiting factor to increased sensitivity is the chemical background noise; this can be coveniently reduced in a variety of ways, including the formation of derivatives of larger molecular weight than *n*-butyl esters or the introduction of electrophoric groups and operation of the mass spectrometer in the negative ion chemical ionization mode.

Coupled with the use of trace enrichment techniques, considerable further improvement in detection limits should be possible. An alternative approach is the use of a post-column triphasic reaction system in which the ion-pairing moiety is bound to a stationary solid support. In this case, the counterion does not enter into the organic phase, which is transported to the moving belt interface, thereby reducing the number of extraneous components that may enter the mass spectrometer. These approaches can be readily automated for routine monitoring of carboxylic acid content in biologically or environmentally derived samples. Work along these lines is currently in progress.

Registry No. $Bu_4N^+OH^-$, 2052-49-5; HCOOH, 64-18-6; CH_3COOH, 64-19-7; CH_3CH_2COOH, 79-09-4; H_2O, 7732-18-5; butyric acid, 107-92-6.

LITERATURE CITED

(1) Tanaka, N.; Budd, M. A.; Effron, M. L.; Isselbaker, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *56*, 236.
(2) Rizzo, A. F. *J. Clin. Microbiol.* **1980**, *11*, 418.
(3) Stack, M. V. *J. Chromatogr.* **1979**, *165*, 103.
(4) Tyler, J. E. *J. Dent. Res.* **1971**, *50*, 694.
(5) Sawyer, C. N.; McCarty, P. L. "Chemistry for Engineering", 3rd ed.; McGraw-Hill: New York, 1978; pp 494–502.
(6) Jellum, E. *J. Chromatogr. Biomed. Appl.* **1977**, *143*, 427.
(7) Tyler, J. E.; Dibdin, G. H. *J. Chromatogr.* **1975**, *105*, 71.
(8) U.S. Public Health Service "Standard Methods for the Examination of Water and Wastewater", 15th ed.; American Public Health Assoc.: New York, 1980.
(9) Bickford, B.; Bursey, J.; Michael, L.; Pellizzari, E.; Porch, R.; Rosenthal, D.; Sheldon, L.; Sparacino, C.; Tomar, K.; Wiseman, R.; Yung, S.; Gebhart, J.; Rando, L.; Perry, D.; Ryan, J. "Preliminary Draft Report Master Scheme for the Analysis of Organic Compounds in Water--Part III: Experimental Development and Results"; U.S. EPA: Athens, GA, 1981.
(10) Henion, J. P. *Anal. Chem.* **1978**, *50*, 1887.
(11) Karger, B. L.; Kirby, D. P.; Vouros, P.; Foltz, R. L.; Hidy, B. *Anal. Chem.* **1979**, *51*, 2324.
(12) Kirby, D. P.; Vouros, P.; Karger, B. L.; Hidy, B.; Petersen, B. *J. Chromatogr.* **1981**, *251*, 175.
(13) Vouros, P.; Lankmayr, E. P.; Hayes, M. J.; Karger, B. L.; McGuire, J. M. *J. Chromatogr.* **1982**, *251*, 175.
(14) Kossa, W. C.; MacGee, J.; Ramachandran, S., and Webber, A. J. *Chromatogr. Sci.* **1979**, *17*, 177.
(15) Hayes, M. J.; Lankmayr, E. P.; Vouros, P.; Karger, B. L.; McGuire, J. M. *Anal. Chem.* **1983**, *55*, 1745.
(16) Tsai, C. -P., Sahil, A.; McGuire, J. M.; Karger, B. L.; and Vouros, P., In preparation.
(17) Manis, F. A.; Geerdink, R. B.; Frei, R. W.; Brinkman, U. A. Th. *J. Chromatogr.* **1985**, *323*, 113.

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